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Growth and physiological responses of young olive trees affected by *Olive Leaf Yellowing Associated Virus*

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Abstract

In this study, some of the relevant biometric and physiological changes that Olive Leaf Yellowing associated Virus (OLYaV) may cause in olive were investigated. The trial was carried out on 32 two-year-old virus-free trees of cv. Frantoio grafted on olive seedlings and grown in 32-l pots. One half of the trees was inoculated with OLYaV in fall 2007. At 6, 18, and 24 months after inoculation, tree height, basal trunk diameter and total leaf number were determined, and leaf area per tree was estimated from a 50-leaf subsample. Twigs of various order were also counted and measured. At the end of the experiment, dry matter partitioning, leaf gas exchange and water potential were also measured. Total twig length and number, trunk diameter and leaf area were significantly reduced by OLYaV, whereas no effect was detected on tree height. Water potential was lower in virus free trees due to a greater leaf area; gas exchange rates were proportional to water status. Trees affected by OLYaV partitioned more dry matter to roots and less to the main stem compared to virus-free trees. The observed growth reductions cannot be explained by differences in water relations or assimilation, but may relate to the sink's ability to attract and/or use assimilates.

Keywords: OLYaV, dry matter partitioning, leaf area, stem water potential, gas exchange

INTRODUCTION

To date, 15 viruses, belonging to 7 different genera, have been isolated from olive trees (Saponari and Savino, 2003; Loconsole et al., 2010.). Most of them were isolated from symptomless plants and only in few cases the presence of the virus was associated with specific symptoms, e.g. the *strawberry latent ringspot virus* (SLRV) which causes severe malformations to fruits and leaves (Marte et al., 1986) and the *olive leaf yellowing associated virus* (OLYaV) which is responsible for yellowing of leaves (Savino et al., 1996).

OLYaV was detected and identified for the fist time in Sicily on 20-year-old olive trees of the local cultivar Biancolilla (Savino et al., 1996). The virus belongs to the family *Closteroviridae* but the genus and species have not been identified yet (Luigi et al., 2010). OLYaV usually shows chromatic alteration of the leaves consisting in a light yellow colouring which extends from the apical area to the border of the leaf, sometimes involving the whole leaf blade. A recent epidemiological survey found a high level of infection (> 60%) on olive orchard of southern Italy (Sicily and Calabria) and in all cases the trees did not show any symptom (Albanese et al., 2003; Faggioli et al., 2005).
Its epidemiology is also unknown, but the high spread of the infection trough the region of southern Italy suggests that its transmission is possible trough a fling vector; indeed the gene sequence of OLYaV has been found in psylla *Euphyllura olivina* (Sabanadzovic et al., 1999), but its role as vector has never been confirmed. Certainly, its spreading in olive

groves is also supported by the use of infected propagation material.

Considering the high level of OLYaV infections in olive groves of southern Italy and often in the absence of symptoms on the trees, we decided to study vegetative growth, assimilation and dry matter partitioning in young olive plants affected by OLYaV.

MATERIALS AND METHODS

The study was carried out using two-year-old virus-free 'Frantoio' olive plants grafted on seedlings, grown in 32-L pots filled with 50% of sterile peat and 50% of sterile pumice. Sixteen plants were inoculated with OLYaV in fall 2007; two bark wedges were collected from an infected plant and budded on the rootstock of the virus free plants. The same operation was made on 16 control plants, using buds coming from virus-free plants, to avoid wound-related (budding) differences between the two treatments. Subsequently, all plants were kept in a greenhouse at an average temperature of 24 °C for four months to allow for spreading of viruses on tissues of inoculated plants. In order to verify the success of virus infection, laboratory assays were performed on phloematic tissue in spring; the samples of inoculated plants were tested by RT-PCR. Subsequently, plants were moved out of the greenhouse and arranged according to a random block design with 16 plants in each of the two treatments and four plants per block.

In summer and fall 2009, stem water potential (Ψ_s) was measured between 12,00 and 1,00 p.m. on four sunlit leaves per plant collected from medium position in the canopy. Leaves were wrapped with aluminum foil two hours before measuring with a Sholander pressure chamber. On the same days and similar leaves, net CO_2 assimilation rate (A) and stomatal conductance (g_s) were measured using a Ciras 1 portable gas exchange system (PP system®) portable gas exchange system (CO_2 and H_2O) connected to a gas exchange chamber (Parkinson Leaf Cuvette). Intrinsic water use efficiency (iWUE, mmol mol^{-1}) was calculated as the relationship between A and g_s.

Plant architecture and vegetative growth were studied separating twigs into (Fig. 1) main twig (inserted on the stem), first order twig (inserted on the main twig) and second order twig (inserted on the first order twig). Twig number, length and number of nodes were determined for each class of twigs. Height and diameter (10 cm above the collar) of the main stem was measured, while leaf area was estimated from digital image analysis of a scanned sample of 50 leaves per plant. All the observation were carried out at 6, 12 and 24 months after virus inoculation.

In fall 2009, plants were removed from their pots and divided into leaves, twigs, stem, and roots (thinner and larger than 2 mm). Portions of each plant organ were oven-dried at 60 °C and partitioning of dry matter was calculated.
Statistical differences ($P < 0.05$) were tested by analysis of variance using Systat procedures (Systat Software Inc., Richmond, CA, USA).

RESULTS AND DISCUSSION

Despite the absence of visible symptoms, OLYaV disease significantly affected plant vegetative growth, dry matter distribution and rates of gas exchange in relation with water status. Lack of visual symptoms, as a result, does not mean that the virus has no effect on plant performance.

In particular, virus infection reduced total twig length and number per plant (Tab. 1), and differences became evident after 12 months from inoculation, especially in first order twigs (data not shown). Main and second order twigs were not affected, probably because they had already formed before infection. The disease also affected main stem diameter and leaf area, but not plant height. In particular, reductions of plant leaf area were due to smaller, rather than fewer leaves in OLYaV plants than in virus free plants (Tab. 1). Overall, OLYaV plants had sparser canopies and lower vigor than virus free plants.

Also, in terms of dry weights, infected plants showed less vigor than virus free plants, and those differences were mainly found in stem weights (Tab. 1). Similar root weights may be explained by pot size and some confinement effect. The same result is evident from shoot/root ratios (reduced by infection) and dry matter partitioning directed more toward the roots in OLYaV plants and more toward the stem in virus free plants (Tab. 2). The differences in stock and distribution of assimilates throughout the plant is responsible for the different balance between canopy and roots of the two groups of plants which is set by the bigger vegetative mass of the canopy produced by virus free plants.

During warmer periods and under well watered conditions, virus free plants had a lower Ψ_s than OLYaV plants and this happens because they must sustain a bigger vegetative mass in comparison with infected plants (Tab. 3). The result is reverted in fall during cooler days. Accordingly, plants under more favorable water status exhibited higher gas exchange rates. Specifically, OLYaV plants exhibited higher g_s, A, E, and WUE during summer, whereas virus free plants in fall (Tab. 3).

CONCLUSIONS

Our results point out that OLYaV affects vegetative growth of young plants acting since the first stages of its replication. In addition, the observed growth modifications cannot be explained by water status and gas exchange alone, but may relate to the sink's ability to attract and/or use assimilates. Despite the lack of visual symptoms, growth modifications determine altered canopy architecture in infected olive plants. For those reasons, careful attention must be paid to the sanitary conditions of plant material when starting a new olive grove or during the productive life of mature groves in order to avoid significant economic losses.

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Tables

Table 1. Biometric measurements in virus-free and OLYaV infected olive plants 24 months after inoculation of the virus. Mean values followed by * indicate significant differences at P < 0.05; ** indicate significant differences at P < 0.01; n.s. indicate non-significant differences.

	Virus-free	Infected	
Twig length (m)	25.5	23.5	*
Number of twigs	102.5	81.4	*
Plant height (m)	231.5	211.5	n.s.
Main stem diameter (cm)	2.08	1.91	*
Total leaf area (cm^2)	124.4	95.1	*
Average leaf area (cm^2)	3.64	2.81	**
Plant dry weight (g)	422.0	344.5	n.s.
roots	105.5	108.8	n.s.
stem	168.5	113.4	**
twigs	67.1	54.7	n.s.
leaves	80.9	67.6	n.s.

Table 2. Dry matter partitioning (%) among various organs of virus-free and OLYaV infected olive plants 24 months after inoculation of the virus. Mean values followed by * indicate significant differences at P < 0.05; ** indicate significant differences at P < 0.01; n.s. indicate non-significant differences.

	Virus-free	Infected	
Shoot/root ratio	3.0	2.2	*
Roots	25.0	31.6	*
Stem	39.9	32.9	*
Twigs	15.9	15.9	n.s.
Leaves	19.2	19.6	n.s.

Table 3. Stem water potential (MPa, Ψ_s), stomatal conductance (mmol H_2O m^{-2} s^{-1}, g_s), net assimilation rate (µmol CO_2 m^{-2} s^{-1}, A), transpiration rate (mol H_2O m^{-2} s^{-1}, E), and intrinsic water use efficiency (iWUE, mmol mol^{-1}) were measured in leaves of OLYaV infected and virus free olive plants in 2009.

		Ψ_s	g_s	A	E	WUE
11 August	Infected	-1.02 ± 0.03	153 ± 17.2	10.4 ± 0.93	2.92 ± 0.30	80 ± 11.3
	Virus free	-1.51 ± 0.10	112 ± 23.8	7.5 ± 1.26	2.26 ± 0.44	77 ± 14.5
18 August	Infected	-0.89 ± 0.06	106 ± 19.5	9.5 ± 0.76	2.11 ± 0.29	116 ± 15.5
	Virus free	-1.12 ± 0.07	78 ± 12.0	6.7 ± 0.69	1.73 ± 0.27	107 ± 20.1
25 August	Infected	-0.78 ± 0.09	188 ± 17.5	13.2 ± 1.00	5.20 ± 0.34	72 ± 3.2
	Virus free	-0.98 ± 0.12	161 ± 22.6	10.2 ± 1.35	4.42 ± 0.48	65 ± 2.5
20 October	Infected	-1.52 ± 0.20	85 ± 16.1	7.0 ± 1.78	1.48 ± 0.24	92 ± 31.3
	Virus free	-1.12 ± 0.14	98 ± 11.8	9.1 ± 0.98	1.73 ± 0.20	99 ± 16.6